embedded in paraffin. Six-μm sections were obtained at the medial mid-condylar segment of the knee in the sagittal plane. The sections were stained with hematoxylin-eosin and safranin-O (SO). The modified Mankin's score was applied for histological evaluation of the cartilage degeneration at two areas (contact area and transitional area which located between non-contact and contact) in tibia. About the calcified cartilage and the non-calcified cartilage in each assessment area, the expression intensity of type II collagen by immunohistochemical analysis and SO staining intensity were measured.
Results: In experimental group, the cyst formation was observed in transitional area only at 8-week, whereas the degeneration was not observed before 8-week and in contact area. This degeneration existed in the non-calcified cartilage, where the increased hypocellularity was observed with extension of re-mobilization period. However, in the calcified cartilage of the same area, the hypocellularity was not observed. At transitional area in experimental group, the SO staining intensity in both non-calcified and calcified cartilage were decreased throughout experimental period in comparison with the control group ($P < 0.05$). Although there was no significant difference between the time points, the intensity was decreased after 6-week in both non-calcified and calcified cartilage. On the other hand, the type II collagen expression intensity in both non-calcified and calcified cartilage did not show significant difference between the experimental and the control group and between the time points. About contact area in experimental group, the decreased SO staining intensity were observed at both non-calcified and calcified cartilage throughout experimental period in comparison with the control group ($P < 0.05$), especially, remarkable reduction of SO staining intensity was observed at 8-week. The collagen II expression intensity did not show significant difference between the experimental and the control group and between the time points, similarly to that at transitional area. The modified Mankin's score at both contact and transitional areas in experimental group was higher than that in the control group throughout experimental periods ($P < 0.01$). However, there was no significant difference between the time points.
Conclusions: Current results showed that the cyst formation would have occurred at non-calcified cartilage between 7 to 8 weeks after remobilization. It might suggest that the cyst formation would occur when degeneration of non-calcified cartilage exceed a certain threshold, however the pathology of cyst formation was not clear in this study.

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THE POSSIBLE ROLE OF VASCULO-MECHANICAL FACTORS IN JOINT PATHO-PHYSIOLOGY

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Purpose: Historically intraosseous pressure (IOP) was found to be raised in early arthritis and osteonecrosis. Steroid use and embolic bone diseases were also associated with a raised IOP. More recent research has suggested that the subchondral region may be important in cartilage nutrition. It is probable that high pressures occur in the subchondral region of weight bearing joints. This study was carried out to explore the mechanical and hydrodynamic forces around joints in use. A stepwise approach was used to explore subchondral bone perfusion.
Methods: IOP was measured initially in the cancellous bone of the femoral head, femoral condyle and upper tibia of anaesthetised adult New Zealand White rabbits. A needle was inserted into subchondral bone and connected by a saline filled line to pressure transducers and a chart recorder. Clamps could be placed on the proximal femoral artery and vein. A load of one body weight was applied longitudinally through the limb as required. Similar upper tibial IOP measurements were made in conscious walking volunteers.
Results:
1. Basal intraosseous pressure varied widely from 12-60 mmHg with a cardiac pulse volume (PV) of 3-10 mmHg. In 43 separate studies there was a close correlation between the IOP and its associated pulse volume, $r = 0.801$, $p<0.001$. There was also an underlying respiratory wave (RW).
2. Drugs affected IOP, closely reflecting the systemic circulation pressure changes.
3. Occlusion of the proximal femoral artery causes loss of pressure (IOPa) and pulse volume to virtually nil.
4. Occlusion of the proximal vein causes a rise in pressure (IOPv) with preservation of PV and RW.
5. One body weight load raises IOP with preservation of PV and RW.
6. During arterial occlusion loading caused very little rise in IOP.
7. During venous occlusion loading caused an augmented rise in IOP with preservation of the PV.
8. Perfusion at the needle tip is best understood as a function of IOPv minus IOPa.
9. Simultaneous recordings from the femoral head, condyle and upper tibia during vascular occlusion and loading show the same changes at all sites.
10. Triple recording from the femoral head, condyle and upper tibia during injection of saline shows pressure is transmitted through each whole bone but not across the joint.
11. In man upper tibial pressure during standing, slow walking and fast walking shows large IOP changes of up to 1000mmHg.
Conclusions: IOP is mainly a reflection of arterial supply pressure and not venous back pressure. When IOP is studied combined with alternate proximal arterial and venous occlusion, actual cancellous bone perfusion at the needle tip can be studied. A single IOP measurement in isolation is meaningless. Compartment syndromes could probably be studied in the same way IOPv − IOPa.
IOP is reduced by proximal arterial occlusion and increased by proximal venous occlusion and physical loading.
There is an element of compartmentalisation in each bone. Bones are hydrodynamically separated by joints.
High pressure fluctuations arise in subchondral bone during weight bearing activity. There may be protective anatomical modifications of the subchondral bone microcirculation. Arthritis may be a partly 'vasculo-mechanical' disease. Subchondral bone appears to act as a compressible perfused sponge.

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MECHANOBIOLOGICAL RESPONSE OF ARTICULAR CARTILAGE SUBJECTED TO SIMULTANEOUS COMPRESSION AND SLIDING

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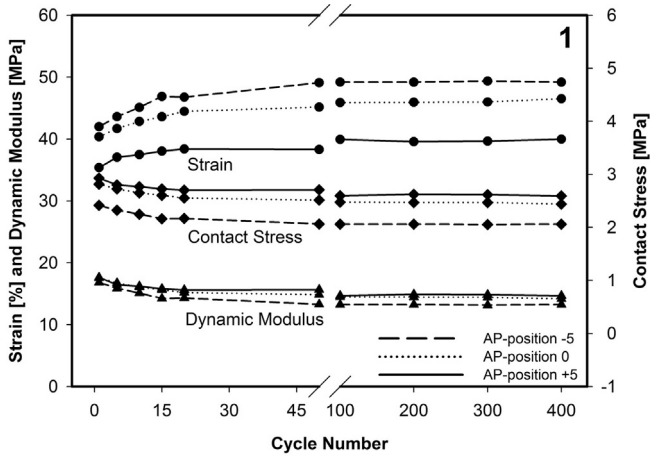
Purpose: The biological and mechanical response of articular cartilage to stationary compressive and shear forces has been extensively studied. In physiological conditions however, joint locomotion involves continuously changing contact areas over the tissue surface. Such migrating contacts play an important role for the durability and wear-resistance of the tissue. By allowing the contact area to migrate over a cartilage surface, stress and strain become dynamic parameters depending on the sliding load applied and the contact location. In this study, we investigate the mechanobiological response of articular cartilage to the application of different axial loads at a physiological sliding velocity. We hypothesized that increasing axial load would lead to an up-regulation of genes associated with extracellular matrix remodeling.
Methods: Fresh mature bovine femoral condyles were removed from the knee and mounted into our custom designed cartilage-sliding machine. An axial load was applied and displacements in x-(anterior-posterior, AP) and y-(proximal-distal, PD) directions measured. The cartilage surface was mapped by repeatedly lowering a delrin indenter ($r = 12.7$ mm) onto the surface. A stress-strain curve could then be obtained for each location to determine the surface geometry and mechanical properties. Sliding ($\pm$ 36 mm amplitude) at 10 mm/s speed and one of 4 normal forces (12, 24, 36 and 48 N) was applied for 400 cycles (48 minutes). Experimental and unloaded (control) samples were harvested and incubated for 1h before analyzing for gene expression by RT-qPCR. The bone surface was also mapped to determine the thickness of the cartilage. For each specimen harvested, strain, stress and dynamic modulus after 400 cycles were calculated according to Hertzian theory of elastic deformation. A linear regression model was used to correlate strain, stress and dynamic modulus with gene expression values of the following genes: collagen type IIa, aggrecan, fibronectin, sox-9 and MMP-3.
Results: All condyles undergo creep deformation, reaching "dynamic equilibrium" after 15-25 cycles of loading (Figure 1).

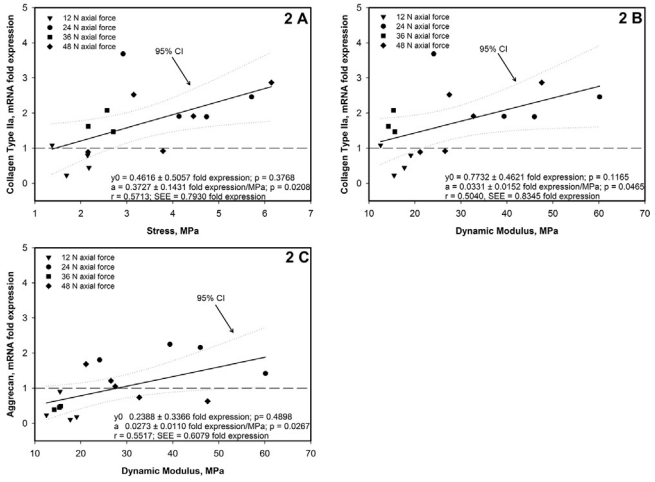


The application of four axial forces led to a variety of strains, contact stresses and dynamic moduli depending on the thickness of the tissue and its mechanical properties. The regression coefficients for all genes correlated with the mechanical parameters can be found in Table 1.

Table 1

Mechanical Parameter	Gene *	r	SEE	y0 *	p-value	a **	p-value
Strain [%]	**MMP-3**	0.3212	0.8666	1.8626	**0.0215**	-0.0288	0.2251
	Fibronectin	0.4627	1.1136	3.4222	**0.0024**	-0.057	0.0711
	Collagen IIa	0.2779	0.9282	0.8778	0.2739	0.0263	0.2973
	Aggrecan	0.1594	0.7196	1.3264	**0.0435**	-0.0114	0.5555
	Sox-9	0.167	0.7686	0.6107	0.3549	0.0128	0.5365
Contact Stress [MPa]	**MMP-3**	0.4919	0.7967	1.9799	**0.0016**	-0.3039	0.0529
	Fibronectin	0.2297	1.2225	1.067	0.1927	0.1948	0.392
	Collagen IIa	0.5713	0.793	0.4616	0.3768	0.3727	**0.0208**
	Aggrecan	0.4186	0.662	0.3124	0.4716	0.206	0.1066
	Sox-9	0.097	0.7758	0.8303	0.1155	0.0511	0.7208
Dynamic Modulus [MPa]	**MMP-3**	0.3933	0.8413	1.6571	**0.0032**	-0.0245	0.1318
	Fibronectin	0.3736	1.1652	0.8331	0.2175	0.0319	0.1541
	Collagen IIa	0.504	0.8345	0.7732	0.1165	0.0331	**0.0465**
	Aggrecan	0.5517	0.6079	0.2388	0.4898	0.0273	**0.0267**
	Sox-9	0.1463	0.7711	0.7855	0.0871	0.0078	0.5888

The analysis revealed significant positive correlations between contact stress and dynamic modulus with collagen IIa and for dynamic modulus with aggrecan (Figure 2).



Conclusions: This study found that a short-term application of simultaneous compression and sliding onto articular cartilage resulted in substantial location-dependent changes in mechanical parameters of the tissue. Both the applied force and intrinsic mechanical properties differed along the curvature of the condyle, leading to varying mechanical responses. Chondrocytes were subjected to different strains and stresses depending on their location, and the mechanical properties of their surrounding matrix, resulting in altered cellular responses in terms of collagen type IIa and aggrecan gene regulation. Additional experiments with different axial loads and/or sliding speeds will be necessary to further quantify the biological response. It is known that anterior cruciate ligament (ACL) deficiencies/tears change AP-translation patterns and accelerate the initiation/progression of OA. Findings of this study might help to identify pathological joint mechanisms leading to OA and provide strategies for prevention and treatment of mechanically induced OA.

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MECHANICAL LOADING OF CARTILAGE EXPLANTS WITH JOINT-SPECIFIC LOADING PATTERNS MODULATES GENE EXPRESSION OF LUBRICIN AND CATABOLIC MATRIX ENZYMES

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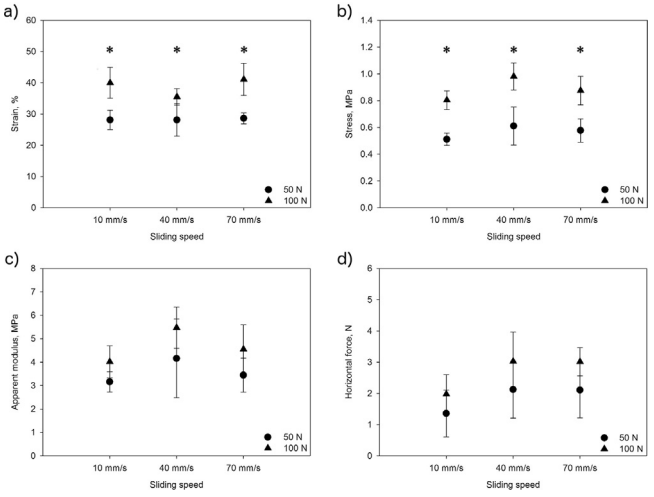
Purpose: Excessive mechanical loading can lead to damage and loss of matrix components due to metabolic changes by chondrocytes and the synthesis of catabolic enzymes such as MMPs and ADAMTS'. Cartilaginous surfaces, such as the temporomandibular joint (TMJ), spend most of the time in relative motion with a constant translation of the contact zone. From previous tribological studies it is known that these migrating contacts are essential for tissues function. The translation of this knowledge into a Mechanobiological model is however lacking. Recent mechanobiological studies almost exclusively apply relatively simple loading regimens; typically uniaxial compressive and/or shear forces alone or in combination and contact areas are kept stationary. This study was designed to investigate how sliding contact areas affect cartilage mechanobiology in terms of catabolic gene expression.
Methods: Cartilage was obtained from bovine nasal septum (BNS) of young calves. The cartilage was cut into appropriate sizes ($70\times17\times2$ mm^3) (L×W×H). Following a 72 hours equilibration period, the first 10 mm of the cartilage strip were glued to a Plexiglas plate with and placed into a tank filled with culture medium at 37 °C. A cylindrical Teflon indenter (Ø 25 mm) was used to apply a normal force of 50 N or 100 N to the cartilage strip. Three different physiological sliding speeds of 10, 40 or 70 mm/s were applied. The load was cycled for 120 minutes over 50 mm of the specimen and the positions and forces of the indenter in the x- and z-directions recorded. The strain, stress and elastic modulus of the cartilage were calculated continually along the sliding path. Following OA-related genes were analyzed directly after loading, 4 hours and 8 hours later: MMP-3, MMP-13, ADAMTS-4, ADAMTS-5, TIMP-1, TIMP-3 and the gene for lubricin. A multiple linear regression model was used to express the mRNA regulation as a function of eight experimental parameters.
Results: Significantly higher ($p \leq 0.05$) strains and maximum stresses were found for samples loaded with 100 N compared to 50 N (Figure 1a and b). No significant differences were observed for apparent moduli and tangential (shear) forces between 50 N and 100 N. No significant differences in any parameters were found for different sliding speeds (Figure 1a,b,c,d).



For 100N, correlations were found for TIMP-3 (r2 = 0.89), ADAMTS-5 (r2 = 0.73), lubricin (r2 = 0.73) and TIMP-1 (r2 = 0.69). Positive and negative correlations of experimental parameters vary with different genes (Tables 1 and 2).